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Electrochemical telomerase assay for screening for oral cancer

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Abstract

Telomerase has long been known to be a marker for cancer. We have developed a new method of detecting it: the electrochemical telomerase assay (ECTA). We have previously confirmed that the assay is easier to do and more precise than the conventional telomeric repeat amplification protocol, which is currently the most widely used. Here we describe a pilot study made to establish a screening system for oral cancer using ECTA. We evaluated three types of clinical samples obtained from 44 patients with oral cancer and 26 healthy volunteers: exfoliated cells from the whole oral cavity, exfoliated cells from local lesions, and tissue from the lesion itself. The current increase ratio (Δi) obtained by ECTA was significantly higher in the oral cancer group for each type of sampling used. The threshold value for Δi was 19% when calculated by analysis of receiver-operating characteristic curves. Sensitivity and specificity values were 86% and 85% for cells from the oral cavity, 82% and 85% in cells from local lesions, and 95% and 92% in cells from the tumour itself, respectively. There were also no significant differences in sensitivity and specificity associated with age, size of tumour, site of lesion, or degree of malignancy. ECTA therefore seems to be a promising assay for screening for oral cancer.

Keywords: Telomerase; Electrochemical assay; Oral cancer screening

Introduction

Oral functions such as chewing, swallowing, and speech have important roles in daily life, and many patients who have advanced oral cancers resected are left with various dysfunctions and aesthetic problems.[1,2] Methods that enable earlier detection of oral cancer may therefore facilitate better outcomes for them by reducing the amount of resection required.

Telomerase is an enzyme that elongates the vertebrate telomere sequence TTAGGG and is associated with cellular immortalisation. Telomerase activity is present in various human cancers, but it is undetectable in most normal somatic cells.[3,4] Its presence is therefore regarded as a marker for cancer.

The telomeric repeat amplification protocol (TRAP) assay is a method of measuring telomerase activity based on a polymerase chain reaction (PCR), but is time-consuming. We have developed a new method of electrochemical telomerase assay (ECTA) to measure telomerase activity that does not require PCR or subsequent gel electrophoresis.[5–7] For ECTA a sample solution is placed on an electrode with immobilised

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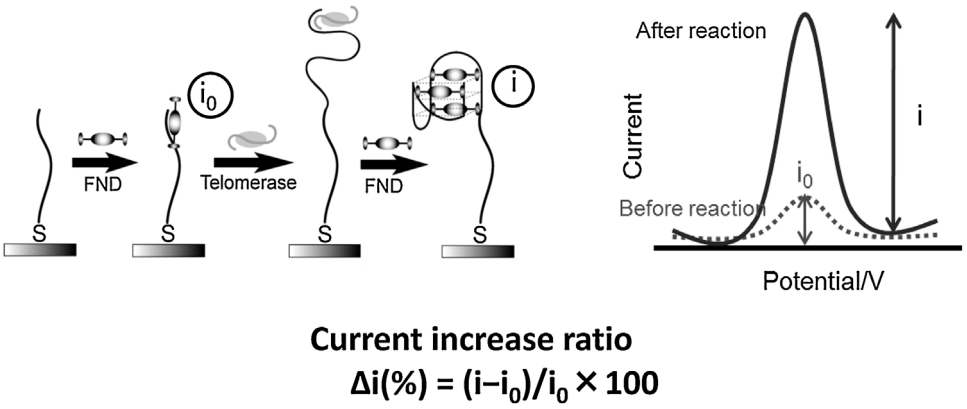

Fig. 1. Diagram of the electrochemical telomerase assay. FND= N,N'-bis[[4-(3-Ferrocenepropionamidopropyl)-piperazin-1-yl]propyl]-naphthalene- 1,4,5,8-tetracarboxylic acid diimide.

telomerase substrate primer, and the current value is measured. If it detects telomerase activity, the telomerase substrate is elongated and the current value increases. The current increase ratio (Δi) reflects the amount of telomerase activity (Fig. 1).

We previously compared the ability of ECTA and TRAP to detect telomerase activity in four cancer cell lines that were derived from oral cancers.[7] ECTA could detect telomerase activity in as little as 8 ng protein (equivalent to about 10 cancer cells), while TRAP required at least 20 times more protein. However, in ECTA the Δi value reached a plateau with relatively few cancer cells present in the sample, so ECTA is a highly sensitive assay for telomerase activity, which is qualitative but not quantitative. We then compared ECTA with TRAP in a clinical trial using both tissue and exfoliated cells scratched from the oral mucosa of patients with oral cancer, and it was vastly superior to TRAP in the detection of telomerase activity from both tissue and cells.

In the present study we aimed to establish an ECTA-based screening system for oral cancer by evaluating the reliability of ECTA in three types of clinical samples from patients with oral cancer.

Material and methods

The study was cross-sectional and done in the Department of Oral and Maxillofacial Surgery, Kyushu Dental University Hospital, Fukuoka, Japan between September 2010 and March 2013. Ethical approval was provided by the ethics committee of Kyushu Dental University (10-19). Samples were obtained from 44 patients with oral cancer and 26 healthy volunteers, all of whom gave informed consent. Patients who had previously been treated for oral cancer by excision, irradiation, or chemotherapy were excluded from the study. All patients were diagnosed histopathologically with squamous cell carcinoma (SCC).

The oral cancer group comprised 24 men and 20 women, mean age 69 (range 34–87) years. Specimens were obtained from SCC of the tongue (n=24), the gingiva (n=16), the floor of mouth (n=3), and the buccal mucosa (n=1). Tumours were sized according to the UICC criteria: T1 (< 2 cm, n=15), T2 (<4 cm but ≥2 cm, n=24), T3 (≥4 cm, n=1), and T4 (includes invasion of nearby tissue, n=4). Histopathological examination identified 27 well-differentiated, 16 moderately differentiated, and 1 poorly differentiated SCC (Table 1). The healthy volunteers were 17 men and 9 women, mean age 47 (range 24–84) years.

Samples were collected in three ways. Exfoliated cells from the whole oral cavity were collected by scratching the entire oral cavity with a sponge-type brush (2 × 2 cm). Five scratches were made on the left and right buccal mucosa, the buccal and lingual gingiva of the upper and lower jaws, and the lingual margin of the tongue. We consider this collection method suitable for use as a self-screening system for patients concerned about oral cancer. Samples of exfoliated cells from local lesions were collected by scratching a lesion with an interdental brush, as is routine for cytodiagnosis. We consider this collection method suitable for use as part of a medical examination for oral cancer by a dentist or other

Table 1
Details of patients with oral cancer.

Variable	Number
Sex:	
Male	24
Female	20
Site:	
Tongue	24
Gingiva	16
Floor of mouth	3
Buccal mucosa	1
Stage of tumour:	
T1	15
T2	24
T3	1
T4	4
Histopathological differentiation:	
Good	27
Moderate	16
Poor	1